The Aryl Hydrocarbon (Dioxin) Receptor: Promiscuity in Activation and Diversity in Response

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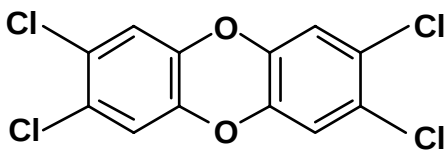
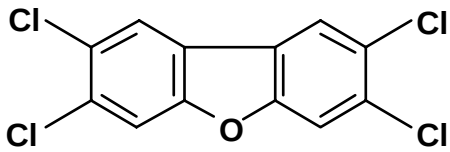
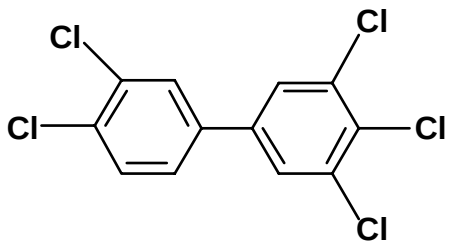
Halogenated Aromatic Hydrocarbons (HAHs)



2,3,7,8-Tetrachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran

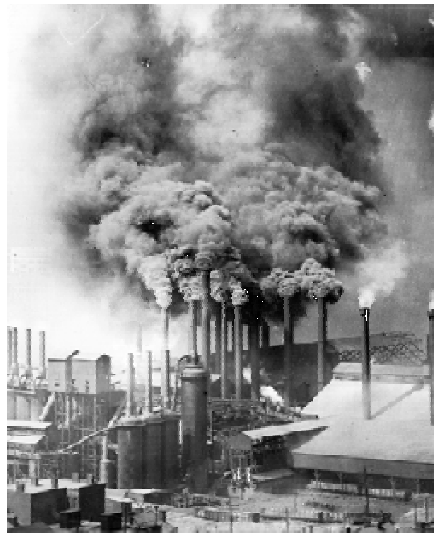


3,4,3',4,'5-Pentachlorobiphenyl

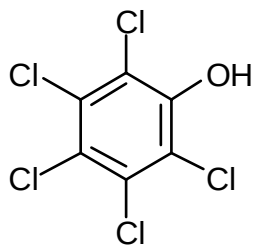
Sources of Dioxins and Related HAHs?

Herbicide Spraying (i.e. Agent Orange)

Food



Combustion



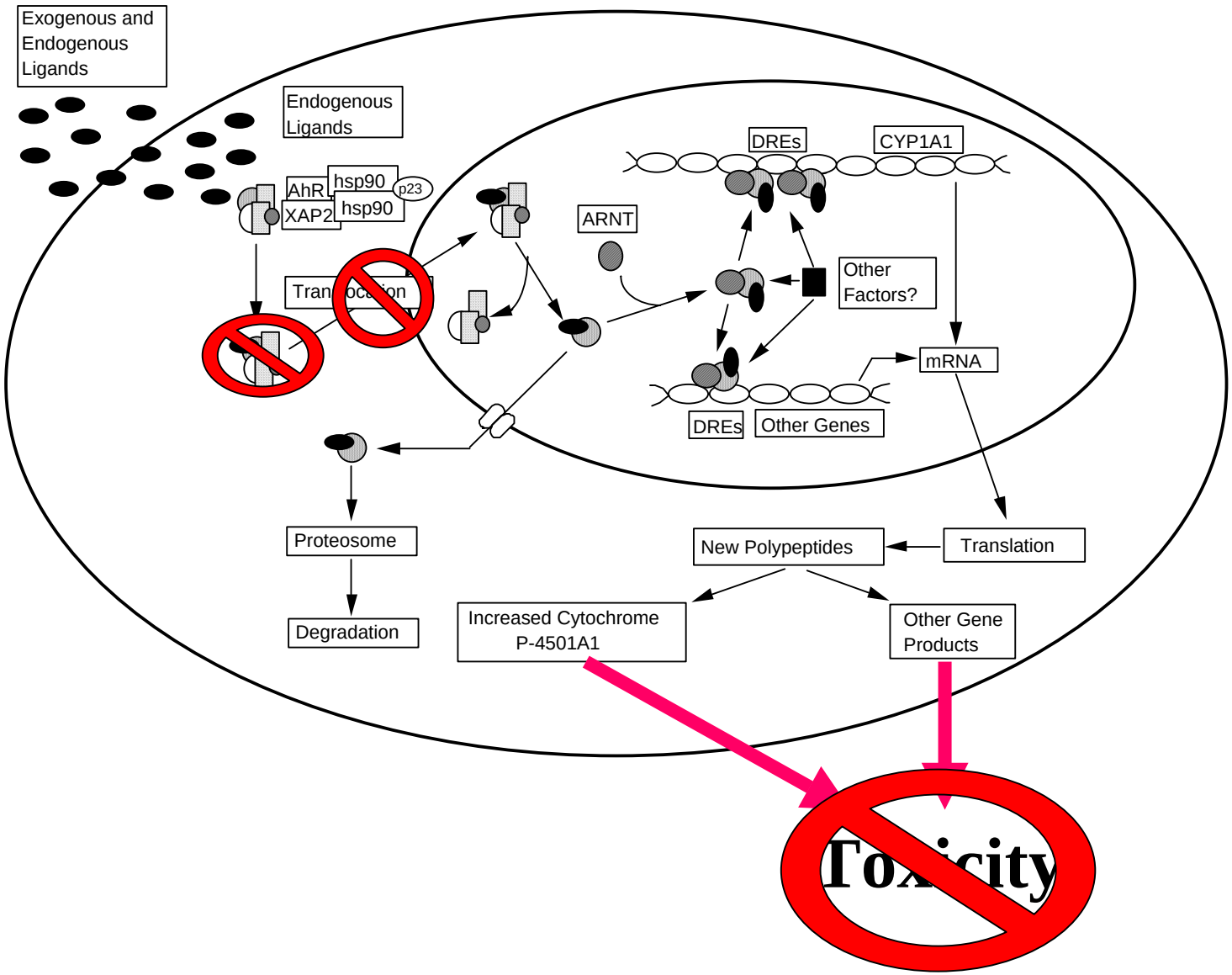
Chlorophenols

Transformers (PCBs)

Spectrum of Toxic and Biological Effects Produced by TCDD in Different Species and Tissues

- Immunotoxicity
- Hepatotoxicity
- Wasting Syndrome
- Dermal Toxicity
- Teratogenicity
- Lethality
- Endocrine Disruption
- Tumor Promotion
- Porphyria
- Induction of Gene Expression (Also Repression)
    - Cytochrome P4501A1/2 and 1B1
    - Glutathione S-Transferase
    - UDP-Glucuronosyl Transferase 1*6
    - Quinone Reductase

AhR Signal Transduction Pathway



QUESTIONS REGARDING AhR SIGNALING

1. **Do all high affinity Ah receptor (AhR) ligands produce the same spectrum of toxic and/or biological effects? HAHs vs PAHs?**

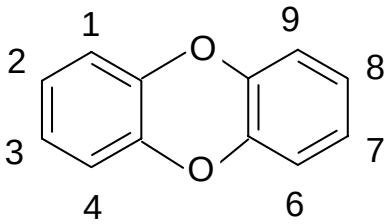
- **What accounts for the differential responsiveness of a cell and animal to AhR ligands?**

- **What is diversity in AhR ligand structure?**

- **What is the significance of AhR ligand promiscuity?**

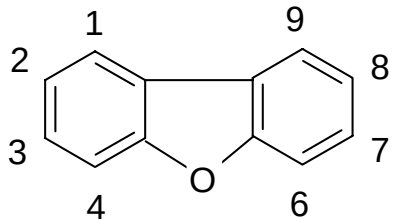
Halogenated Aromatic Hydrocarbon AhR Ligands

PCDDs



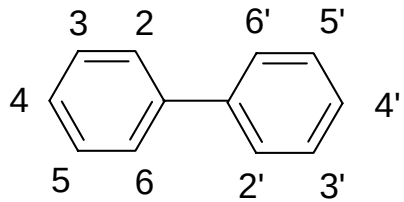
Congeners	TEF
2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDD	1.0
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.01
OCDD	0.0001

PCDFs



Congeners	TEF
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.05
2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.01
1,2,3,4,7,8,9-HpCDF	0.01
OCDF	0.0001

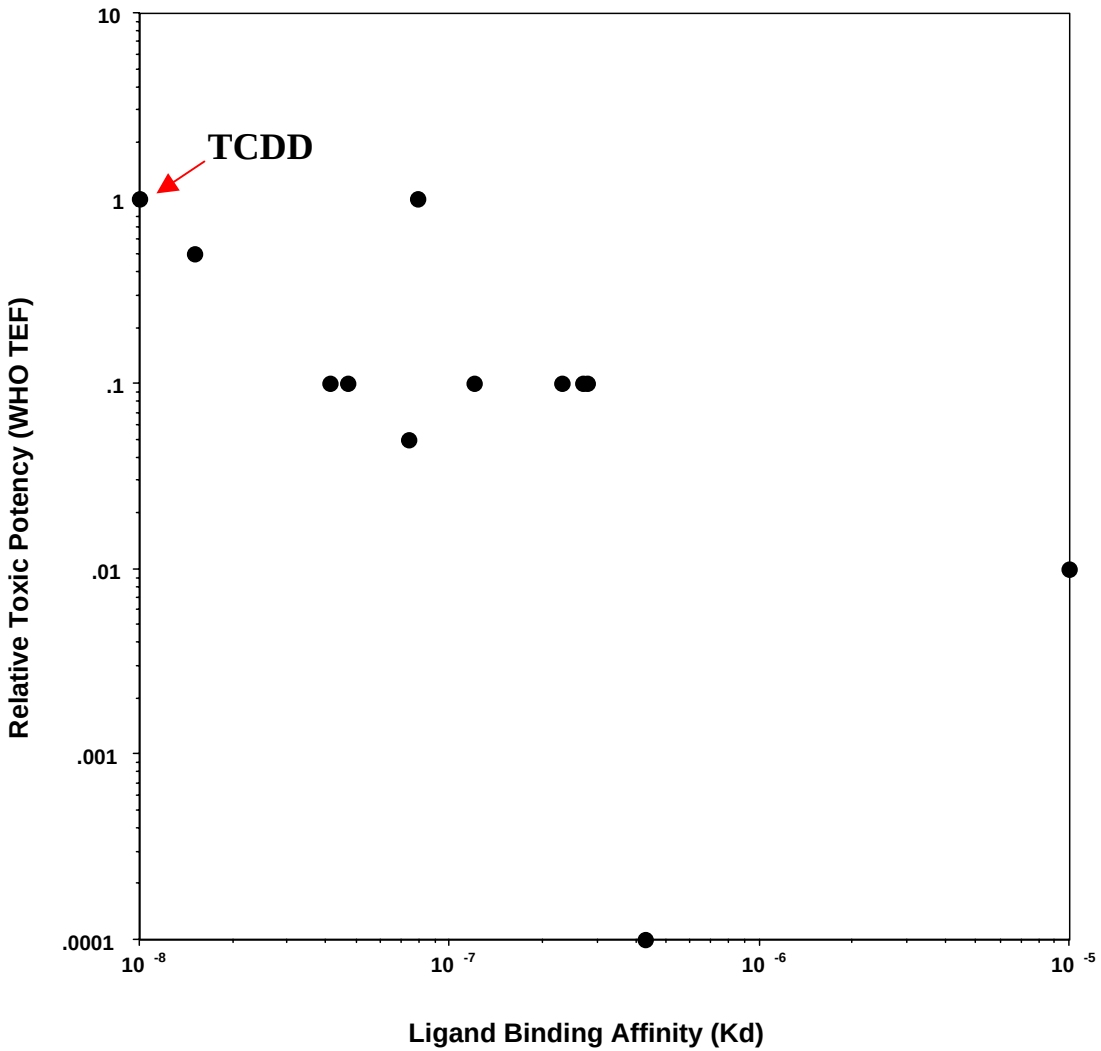
PCBs



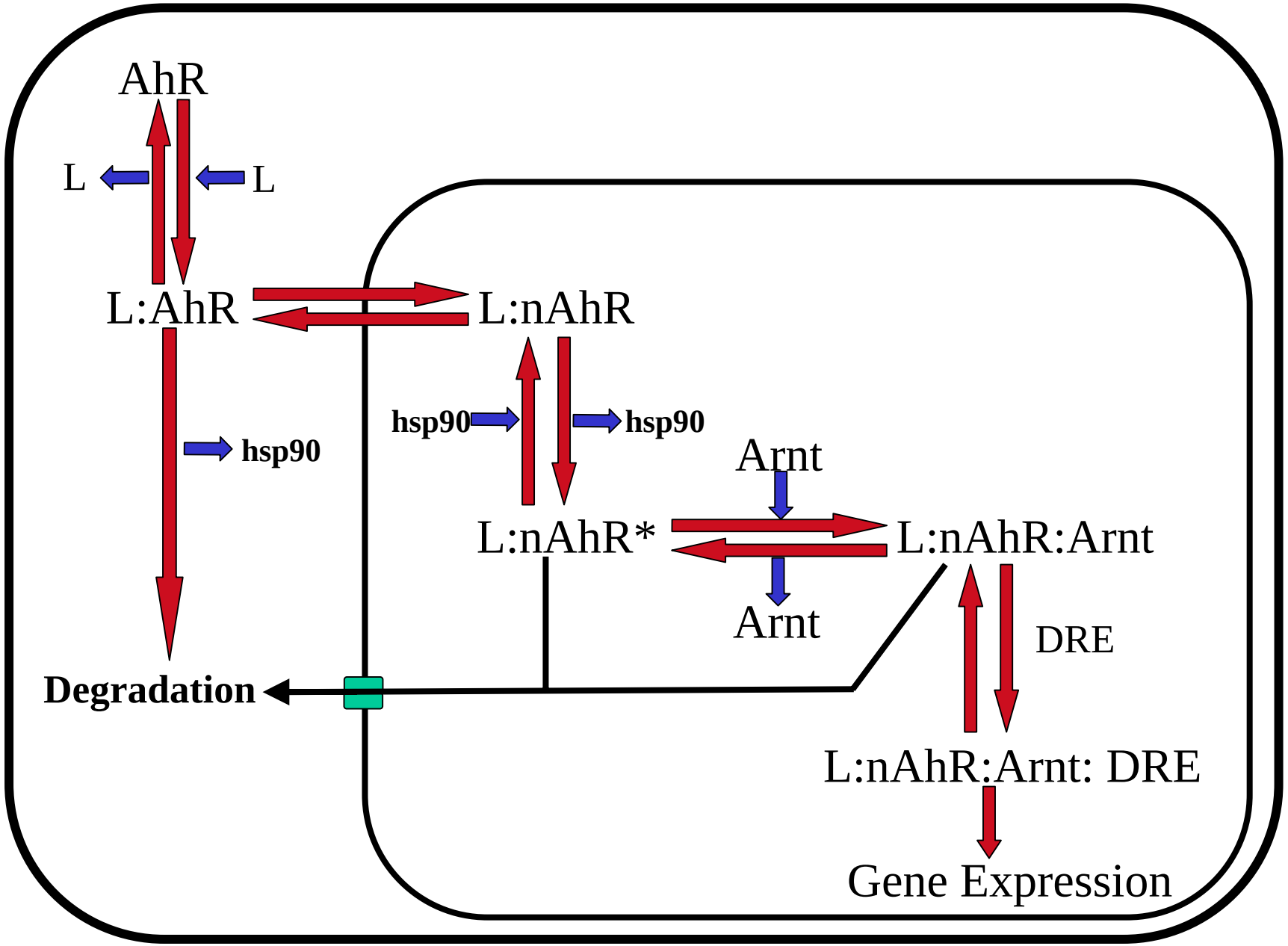
Congeners	TEF
3,3’,4,4’-TCB	0.0001
3,4,4’,5-TCB	0.0001
3,3’,4,4’,5-PeCB	0.1
3,3’,4,4’,5,5’-HxCB	0.01

TEF = Toxic equivalency factor (relative to 2,3,7,8-TCDD)

Comparison of the Ligand Binding Affinity and the Relative Toxic Potency of Selected HAHs

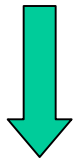


The Mechanism of AhR Action is Dependent Upon a Series of Equilibrium Events

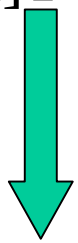


Ligand Dissociation Experiments (Equilibrium Kd ~1nM)

[^{3}H]TCDD (2nM) + cytosolic AhR - 2 hours at 20^{o}C

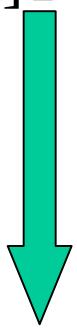


[^{3}H]TCDD:AhR + unbound [^{3}H]TCDD



Dextran-Coated Charcoal to remove free [^{3}H]TCDD

[^{3}H]TCDD:AhR

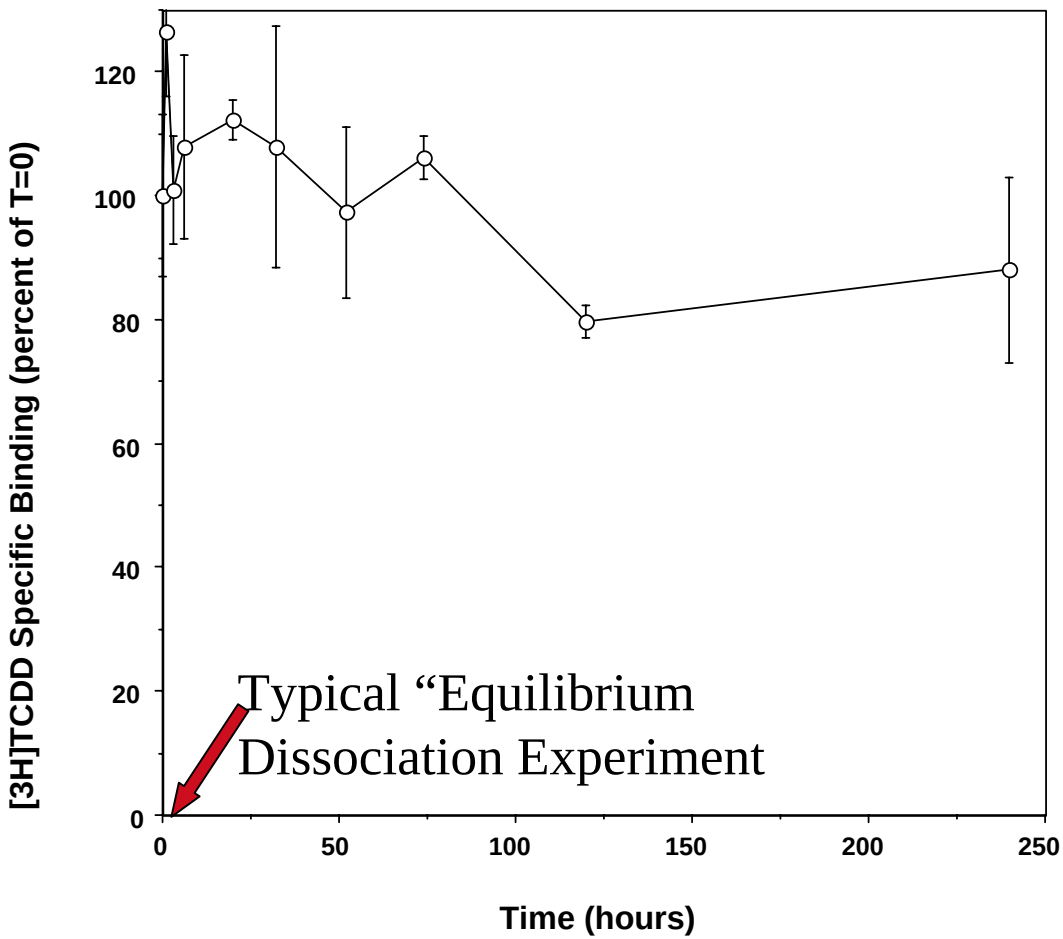


Add unlabeled 2,3,7,8-TCDF (200nM)
measure specific binding at various times

[^{3}H]TCDD:AhR + TCDF:AhR

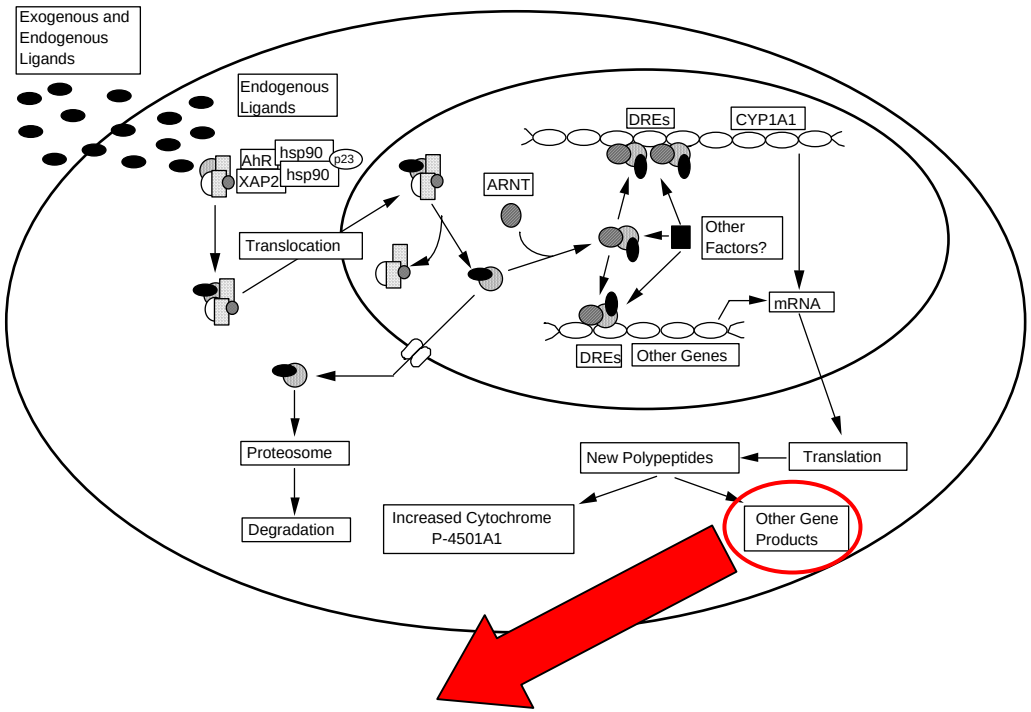
(Decreased specific binding indicates loss of original ligand)

TCDD Binding to the Guinea Pig Hepatic Cytosolic AhR is Essentially Irreversible



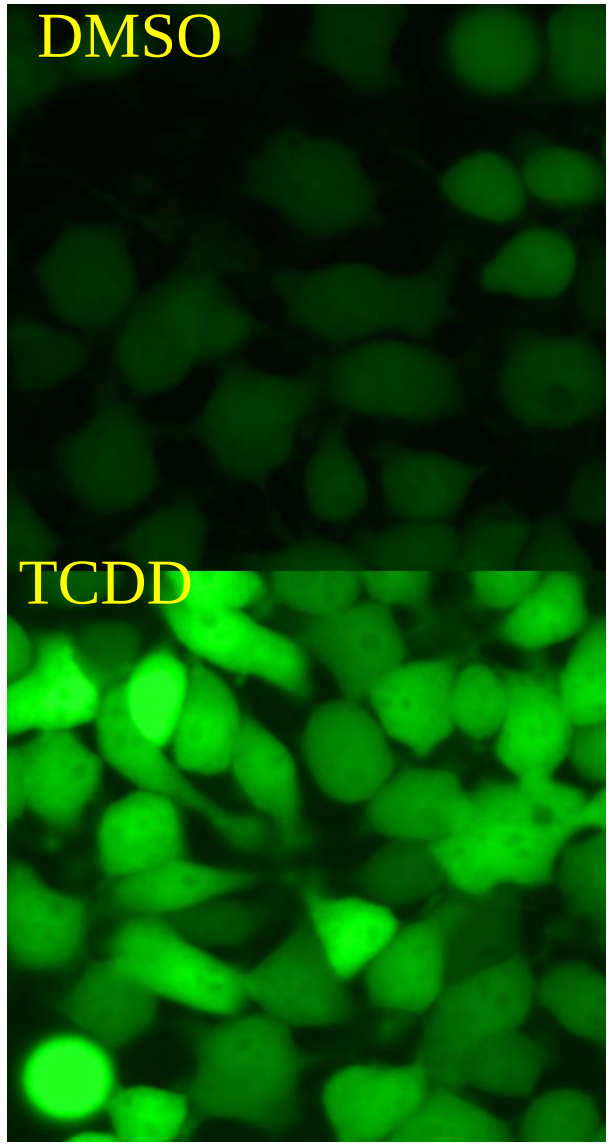
Similar dissociation results were also obtained with other species.

Reporter Gene Induction to Identify and Characterize AhR Agonists and Antagonists

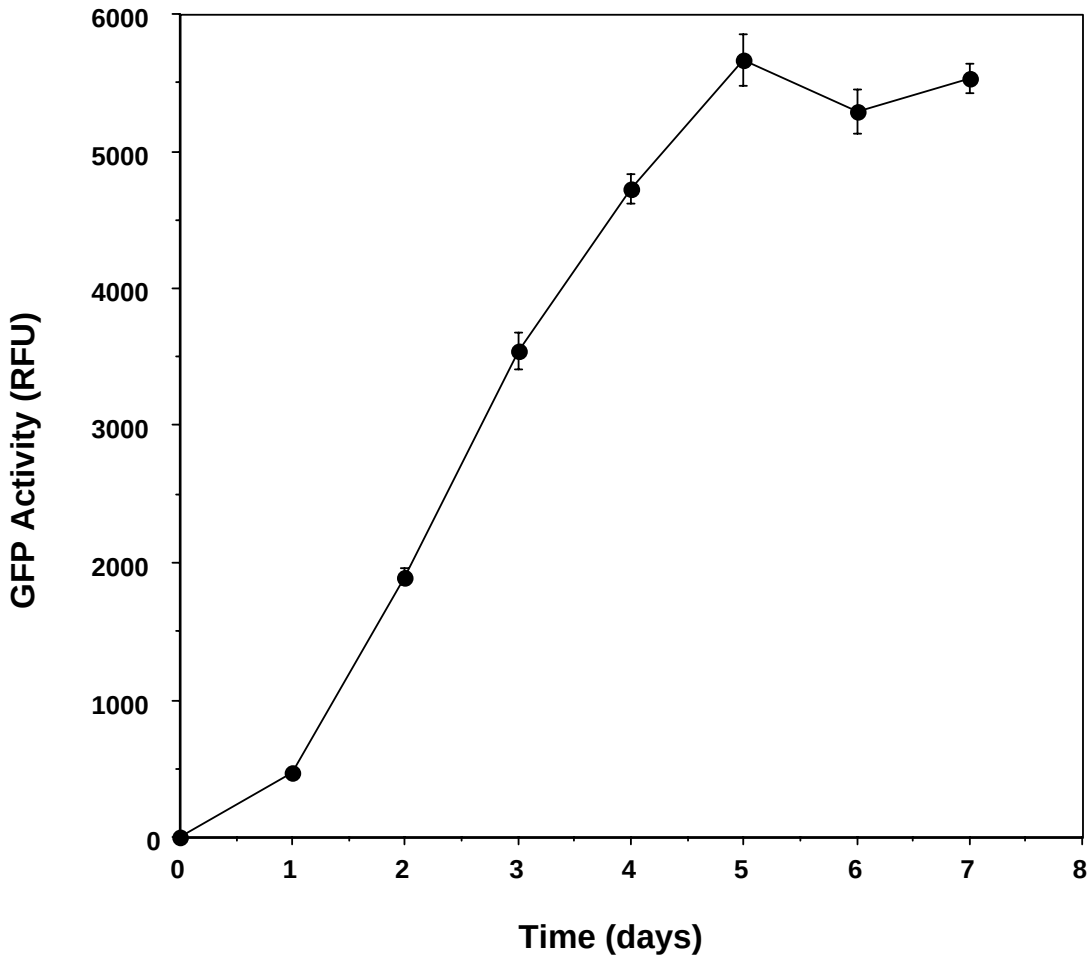


Firefly Luciferase

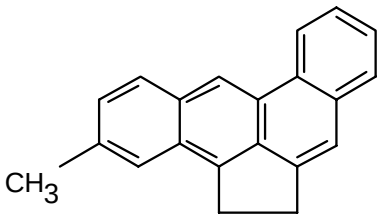
Green Fluorescent Protein (GFP)



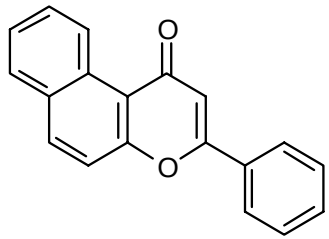
TCDD Induces Persistent Activation of Gene Expression



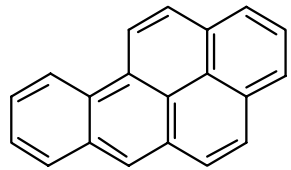
Polycyclic Aromatic Hydrocarbons



3-Methylcholanthrene

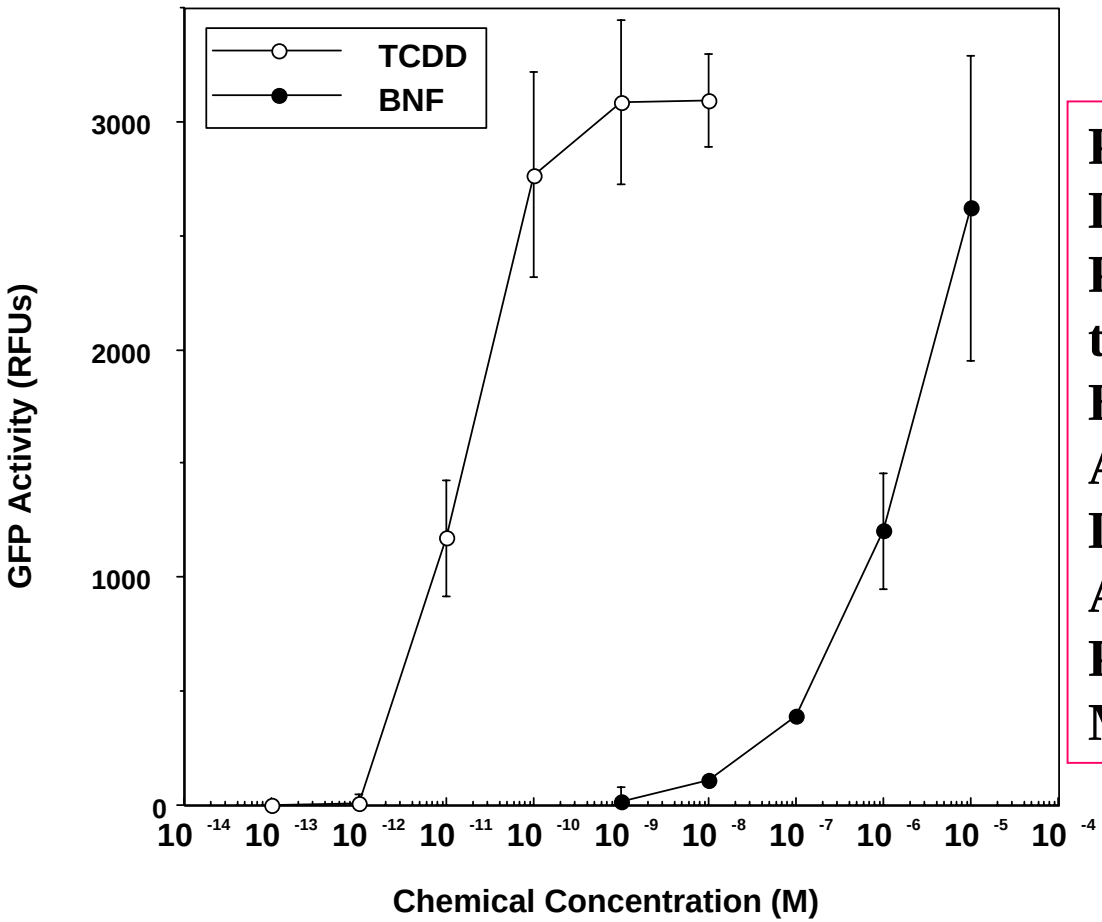


β-Naphthoflavone



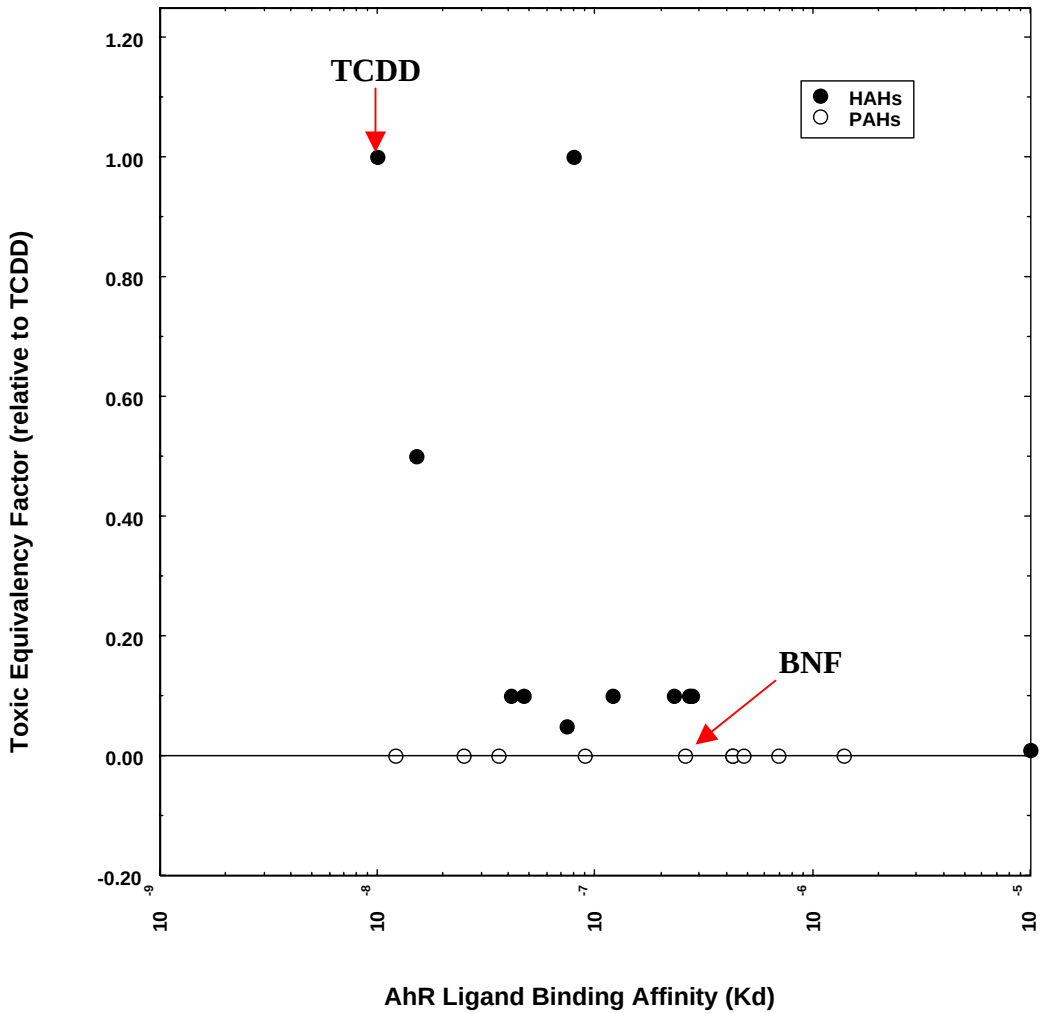
Benzo(a)pyrene

Induction of AhR-Dependent GFP Reporter Gene Induction by TCDD and BNF

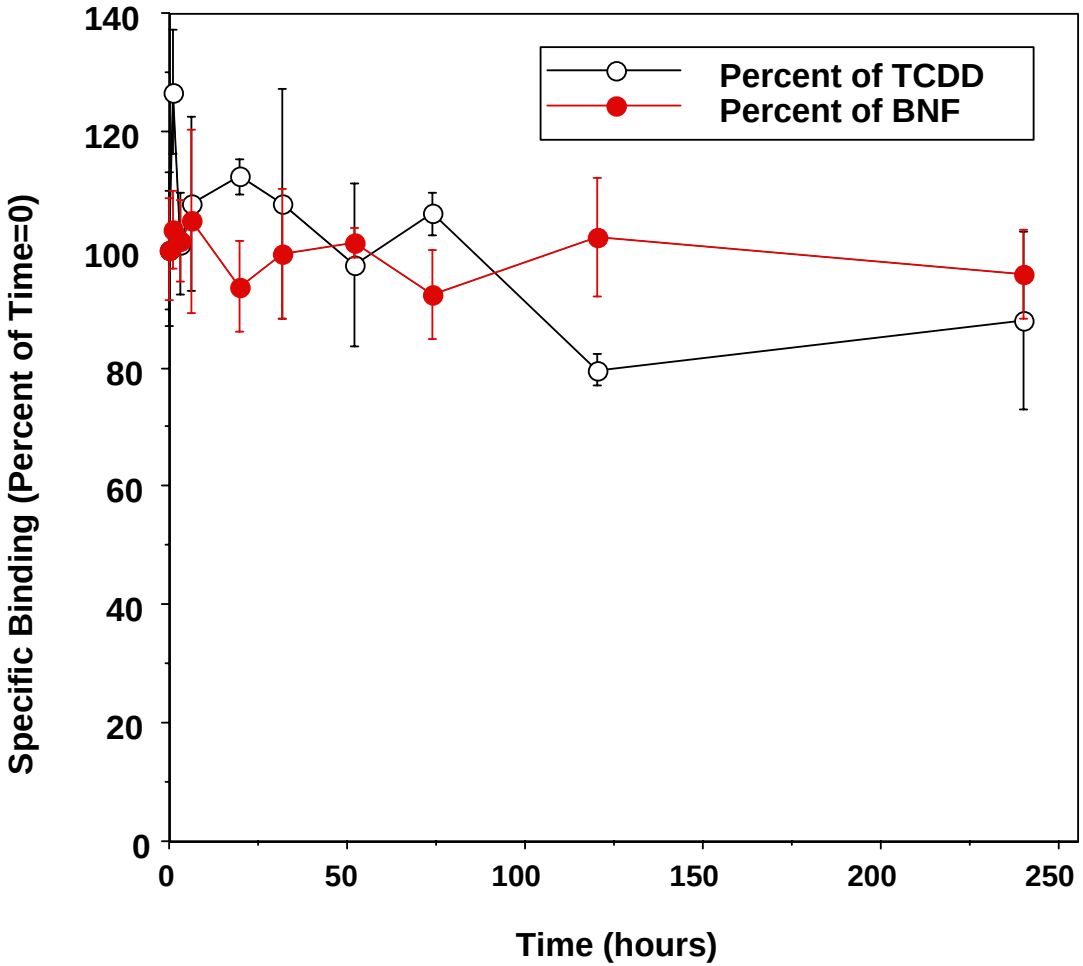


Proposed that the Lower Potency of PAHs was Due to their Lower AhR Binding Affinity that Allows Ligand Dissociation and AhR Inactivation. PAHs are also Metabolically Labile.

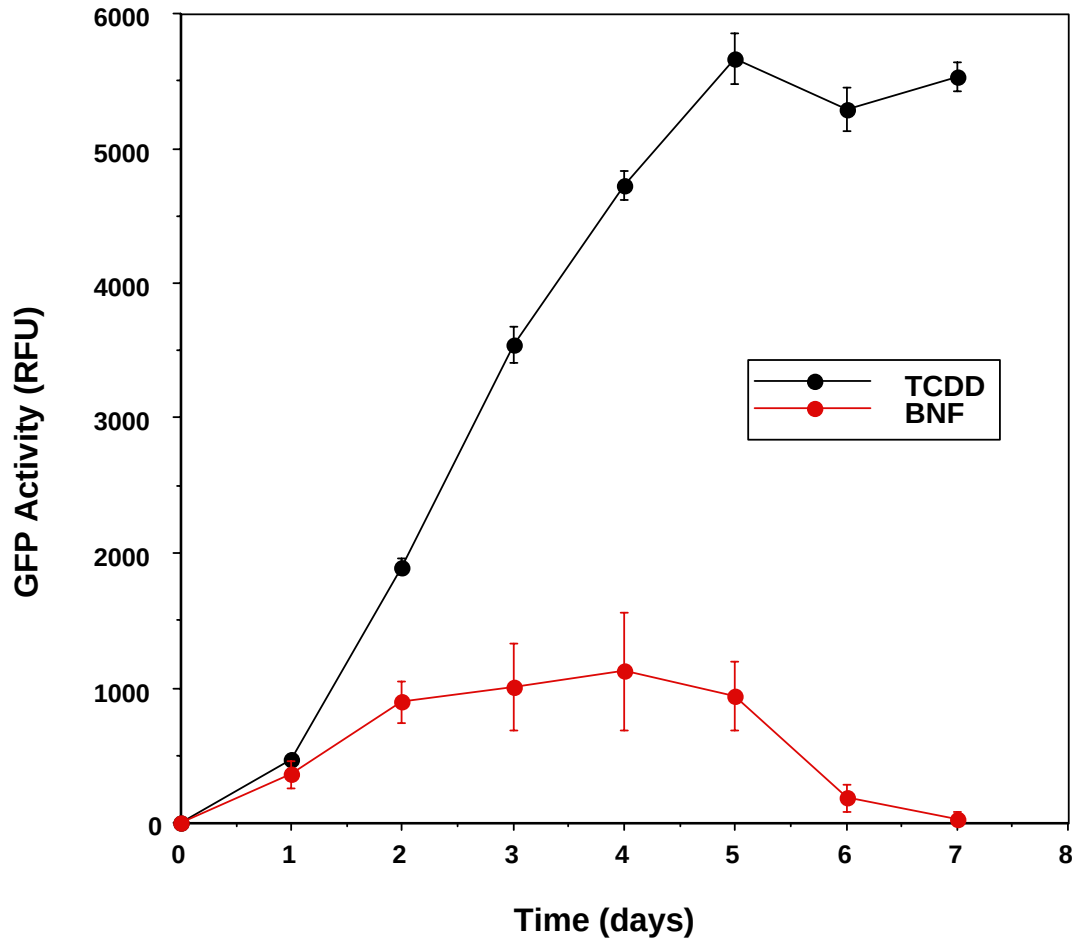
While PAHs have Relative High AhR Ligand Binding Affinity they Produce Little AhR-Dependent Toxicity



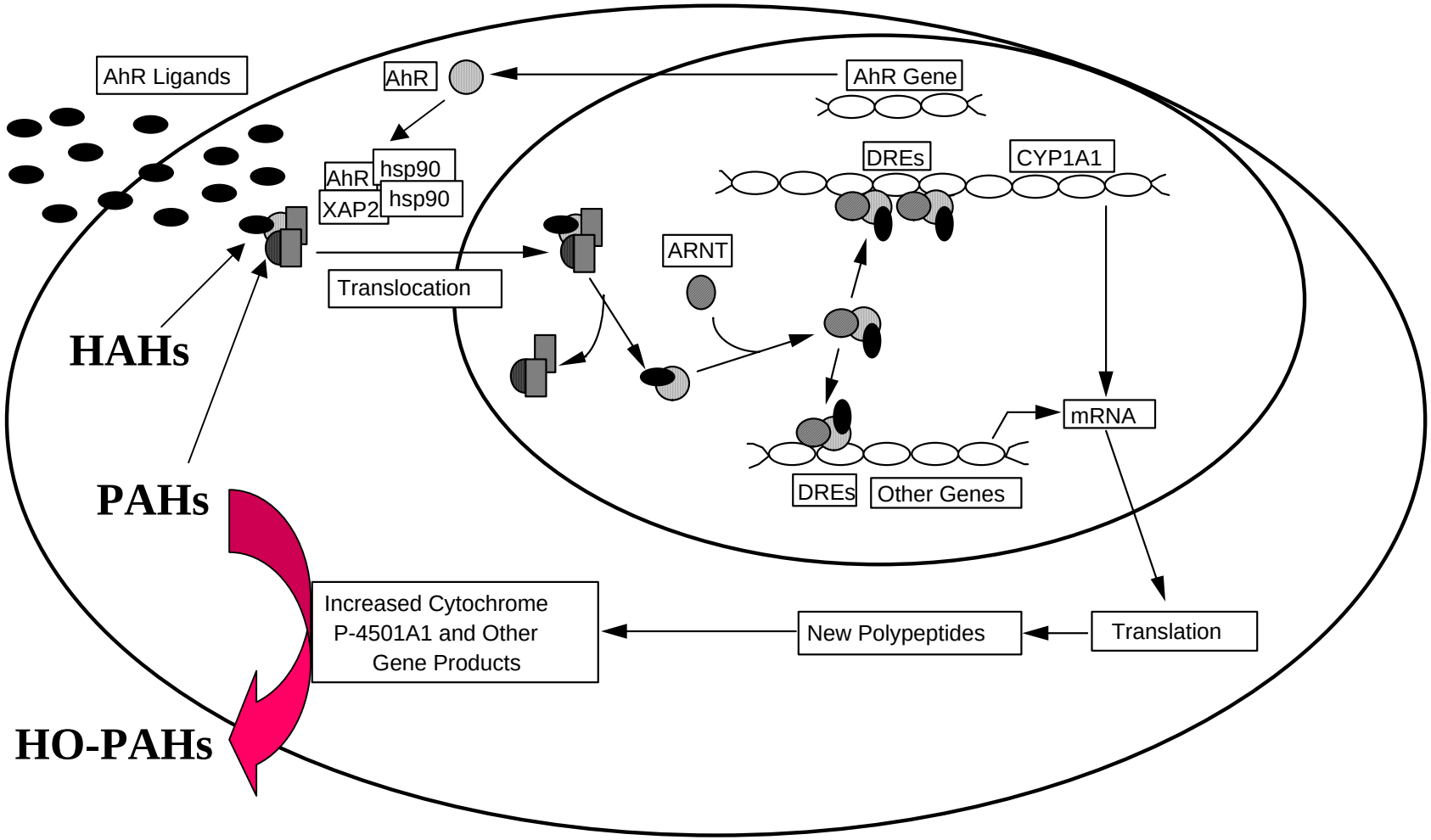
TCDD and BNF are Essentially Irreversibly Bound to the AhR



BNF Induces Transient Activation of Gene Expression

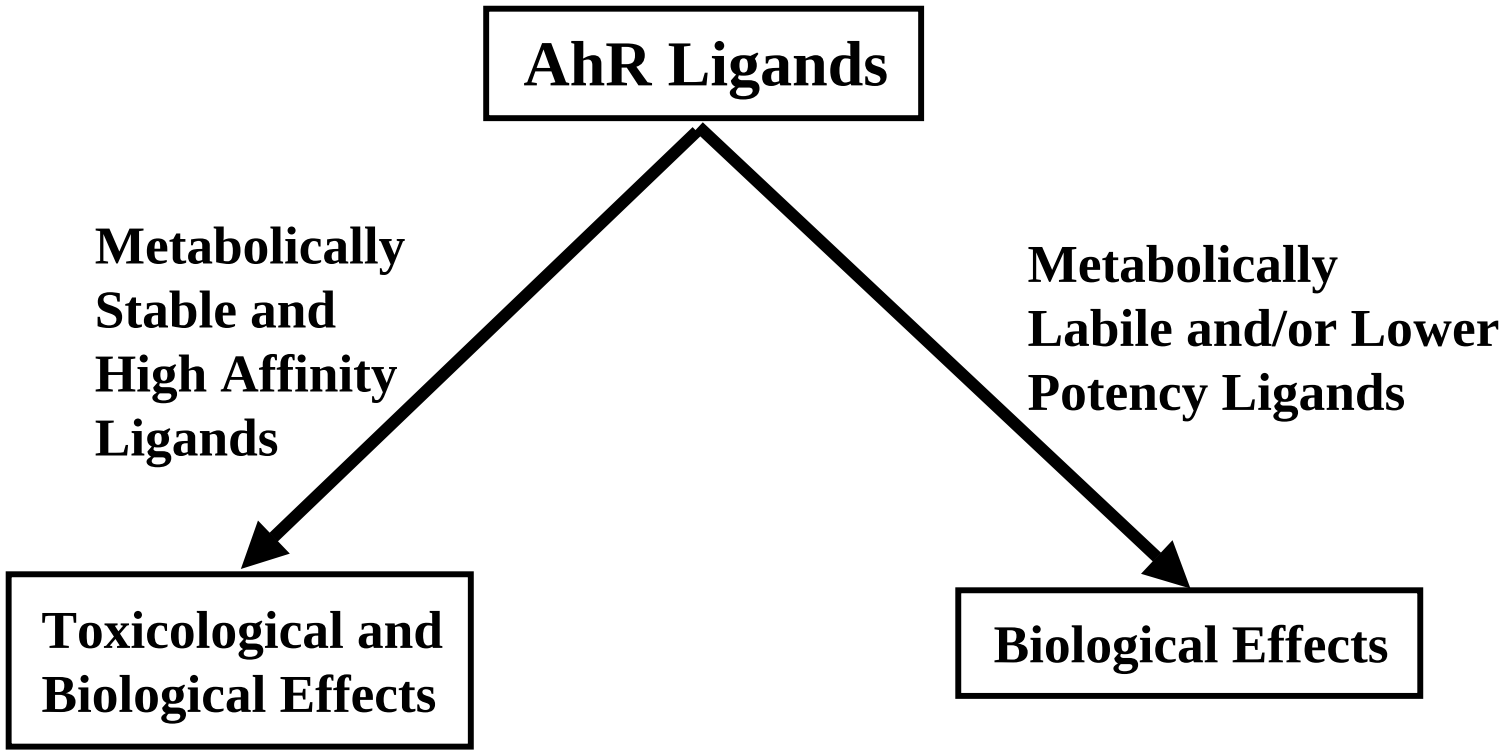


Ah Receptor (AhR) Signal Transduction Pathway



While AhR Binding Affinity is Important, Toxicity of AhR Agonists Appears to be More Dependent upon the Metabolic Stability of the Agonist Which can Lead to the Chronic Activation of Nascent AhR.

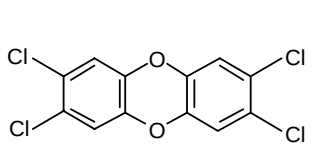
Differential Responses to AhR Ligands



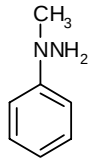
Are AhR Ligands Structurally Similar?

Structural Diversity of AhR Agonists and/or Antagonists

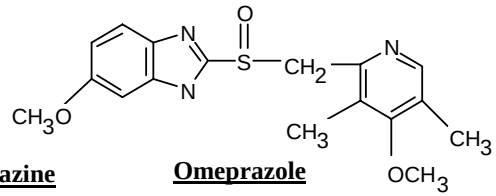
"Nonclassical" Synthetic AhR Ligands and/or Agonists



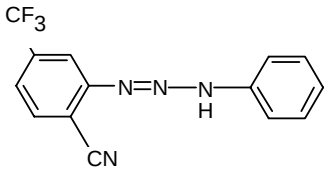
2,3,7,8-Tetrachlorodibenzo-p-dioxin



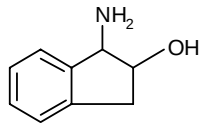
1-Methyl-1-phenylhydrazine



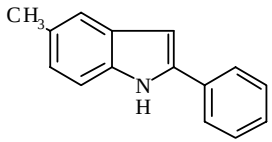
Omeprazole



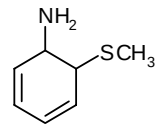
SKF71739



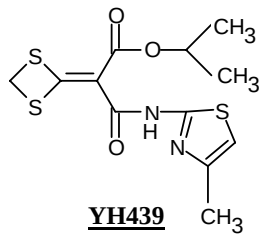
(1S,2R)-(-)-cis-1-Amino-2-indanol



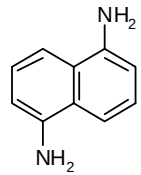
5-Methyl-2-phenylindole



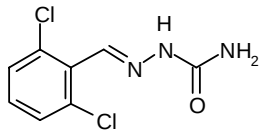
2(Methylmercapto)aniline



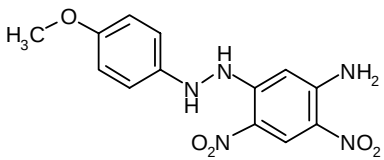
YH439



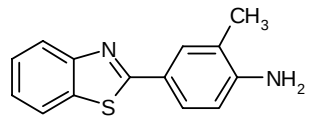
1,5-Diaminonaphthalene



Guanabenz



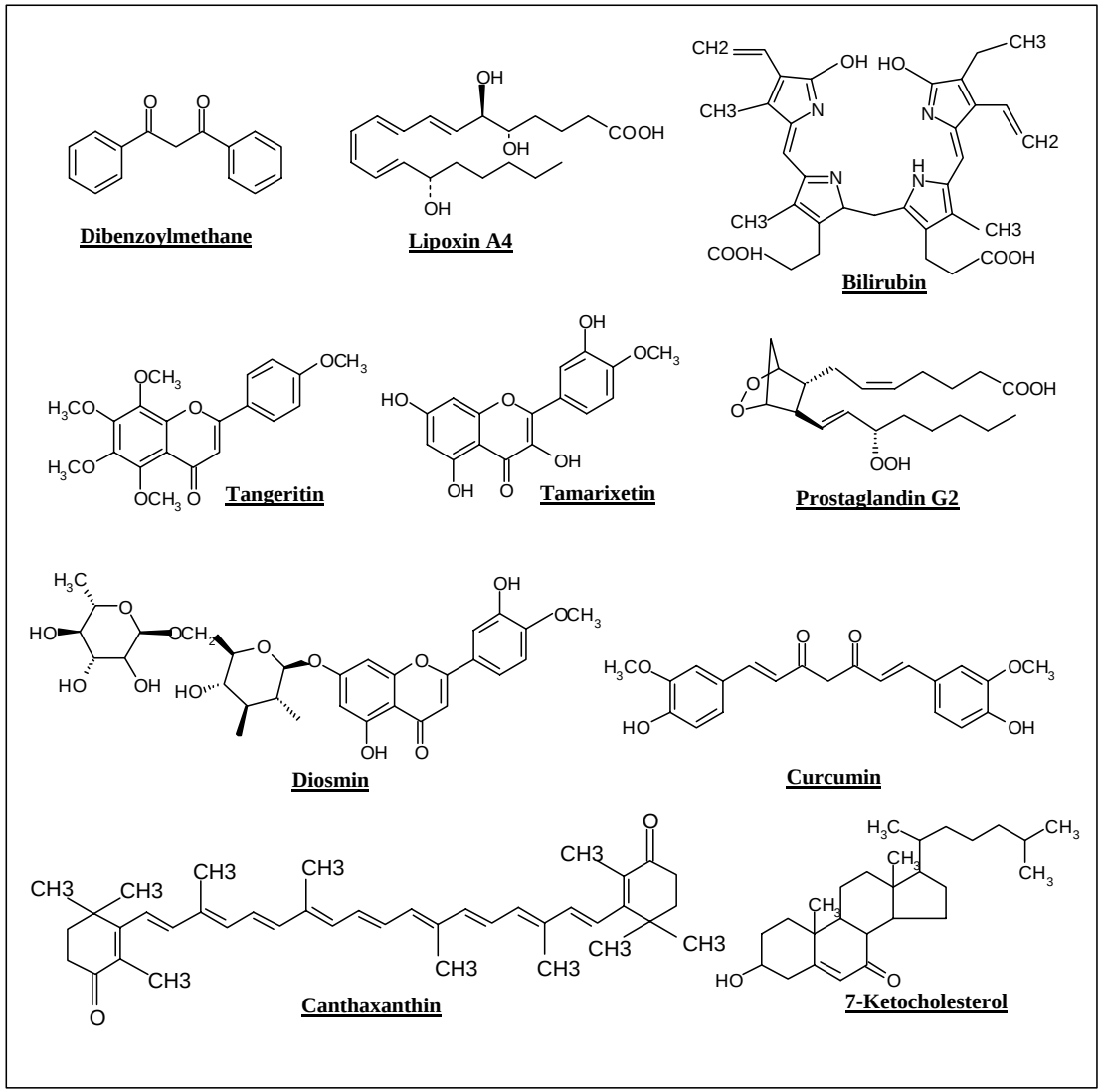
SRN-P2:109,NH2



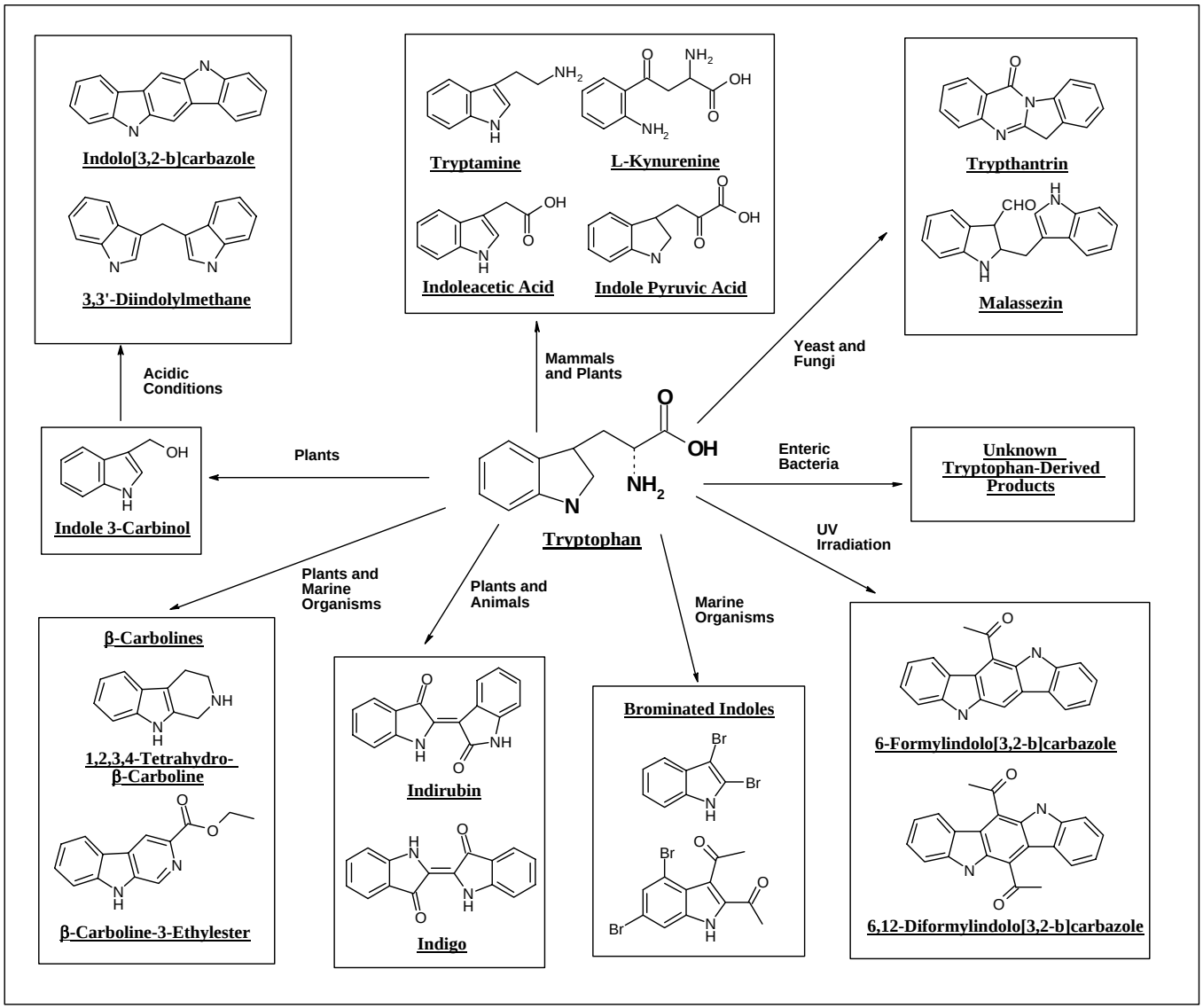
2-(4-Amino-3-methylphenyl) benzothiazole

Structural Diversity of AhR Agonists and/or Antagonists

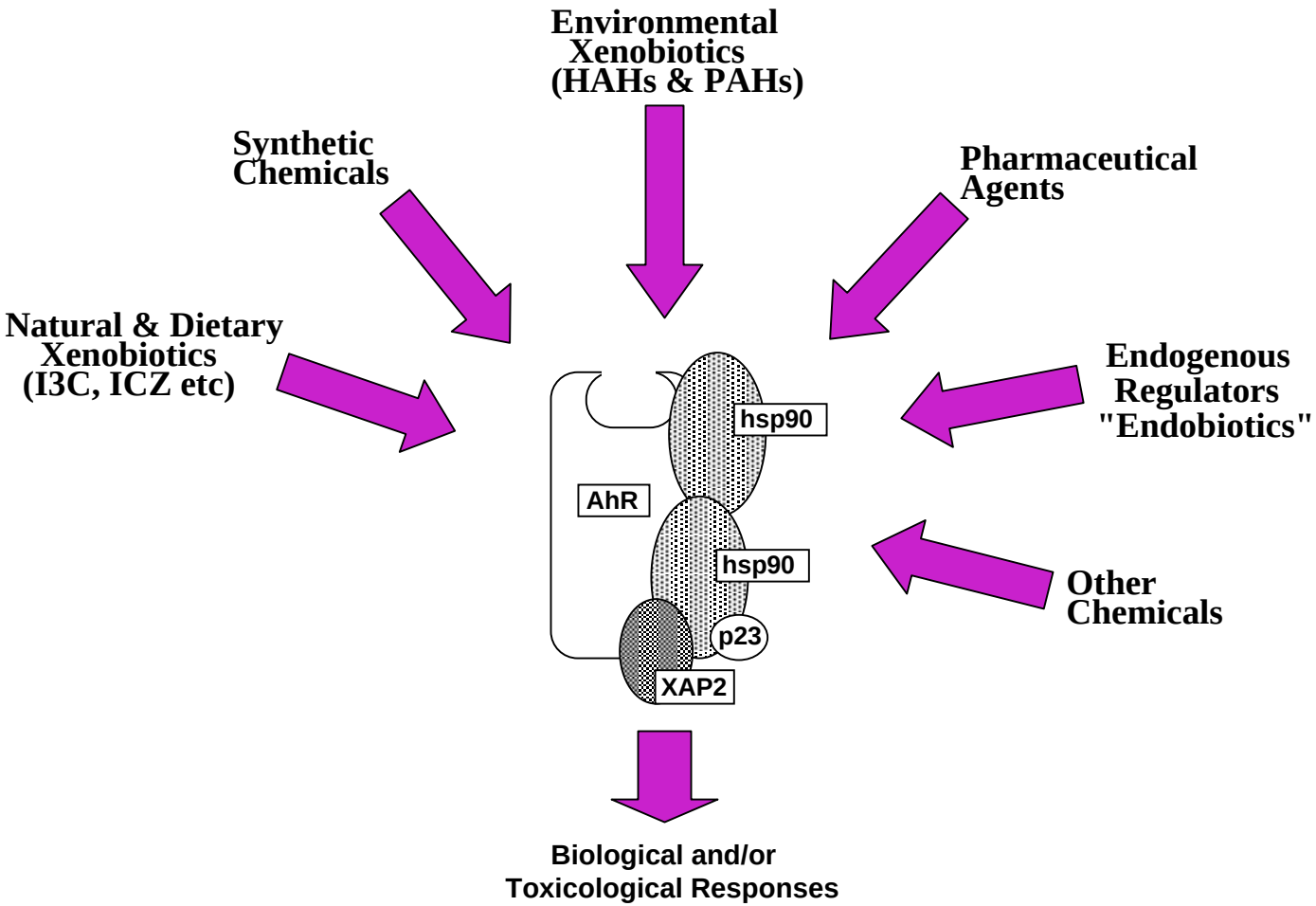
Natural and Endogenous AhR Ligands,Agonists and Antagonists



Naturally-Occurring and Endogenous Indole-Containing AhR Ligands

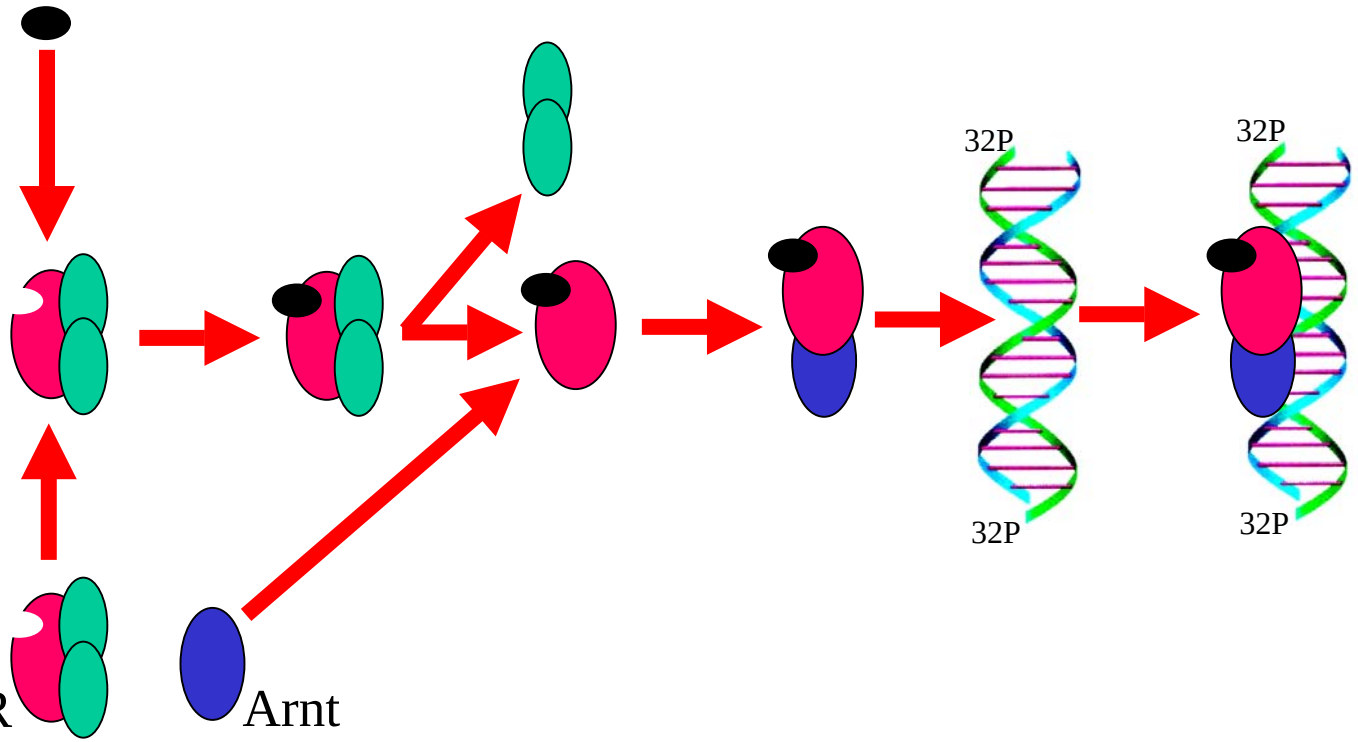


Activation of the Ah Receptor By Diverse Classes of Chemicals



In Vitro DNA Binding Assay for Ah Receptor Agonist Screening

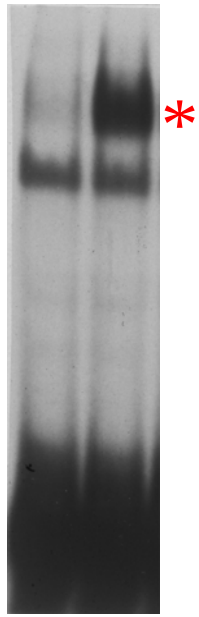
TCDD, pure AhR agonist or a extract containing AhR agonists



Liver Cytosol (AhR & Arnt)

Gel Retardation Assay

TCDD - +



Amount of AhR:DNA complex is directly proportional to the amount of TCDD or other agonist in sample

Simple Extraction of AhR Agonists from Products

1. Sample is weighed and added to a tube.

2. DMSO is added at the following ratio:
   Absorbent products: 1 mg product : 10 μl DMSO
   Nonabsorbent products: 1 mg product : 1.5 μl DMSO

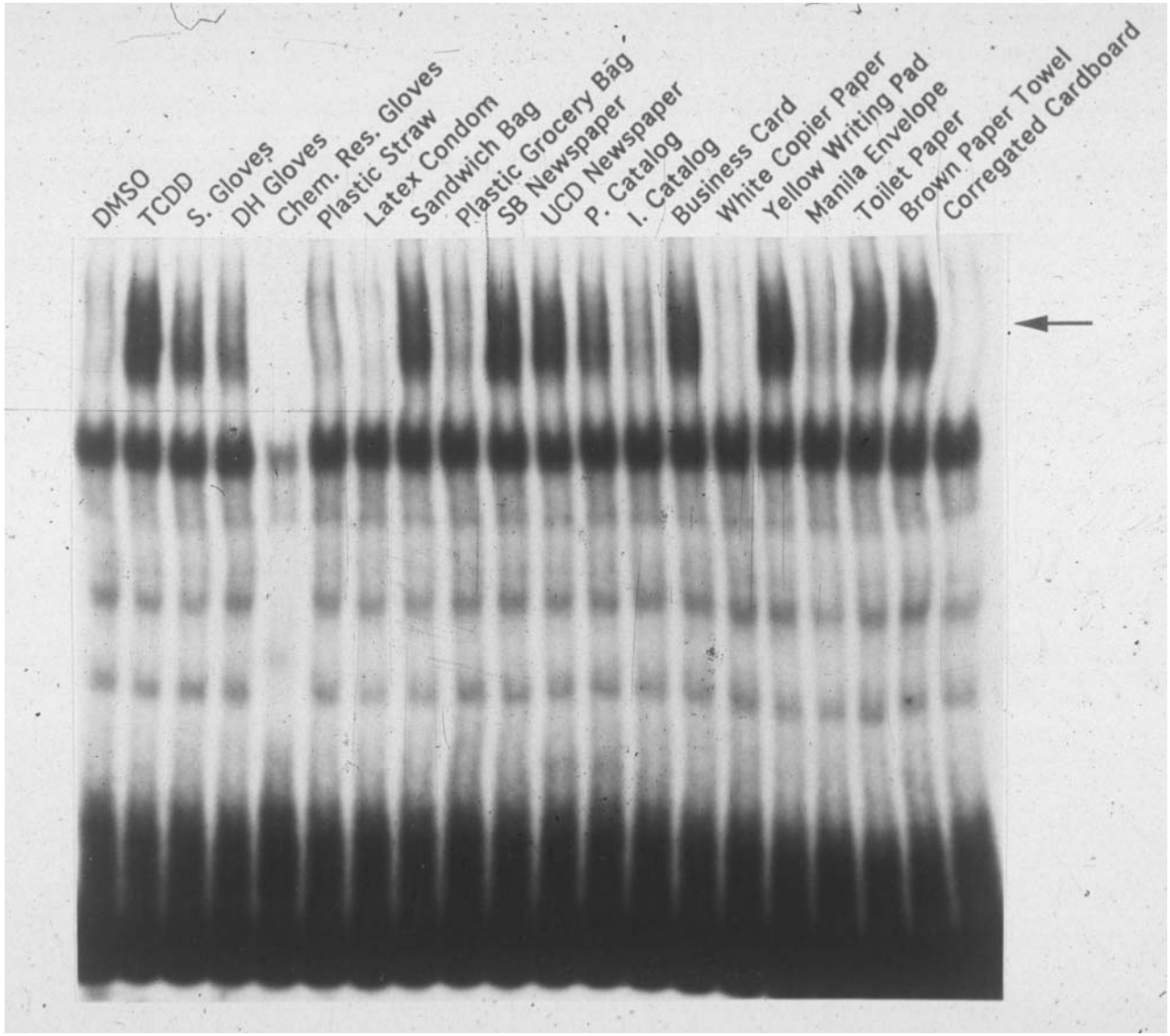
3. Tubes are left overnight at room temperature.

4. DMSO collected from tubes.

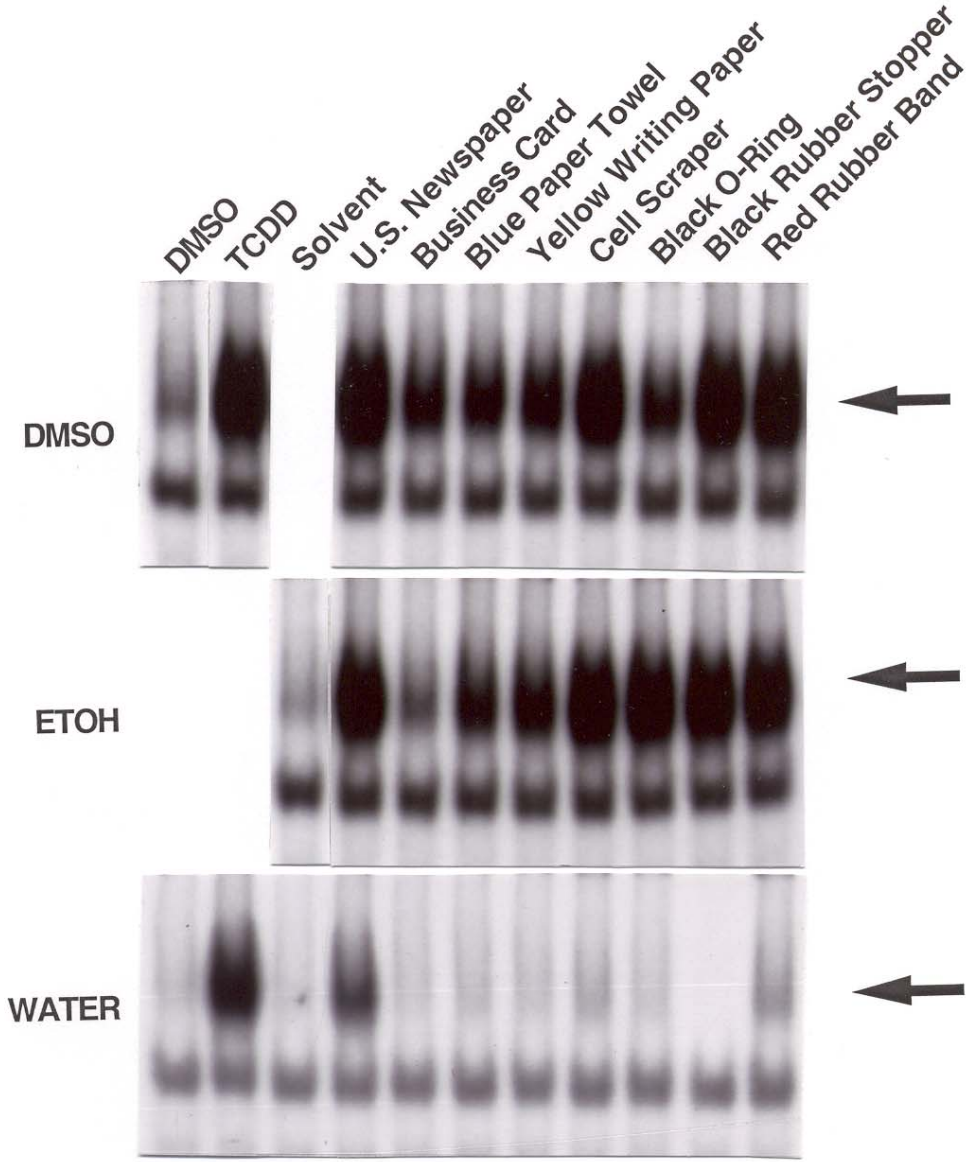
5. Aliquots (1.25 μl) are tested for their ability stimulate AhR-dependent DNA binding by gel retardation analysis.

6. Inducible AhR:DRE complex formation quantitated by Phosphorimager analysis.

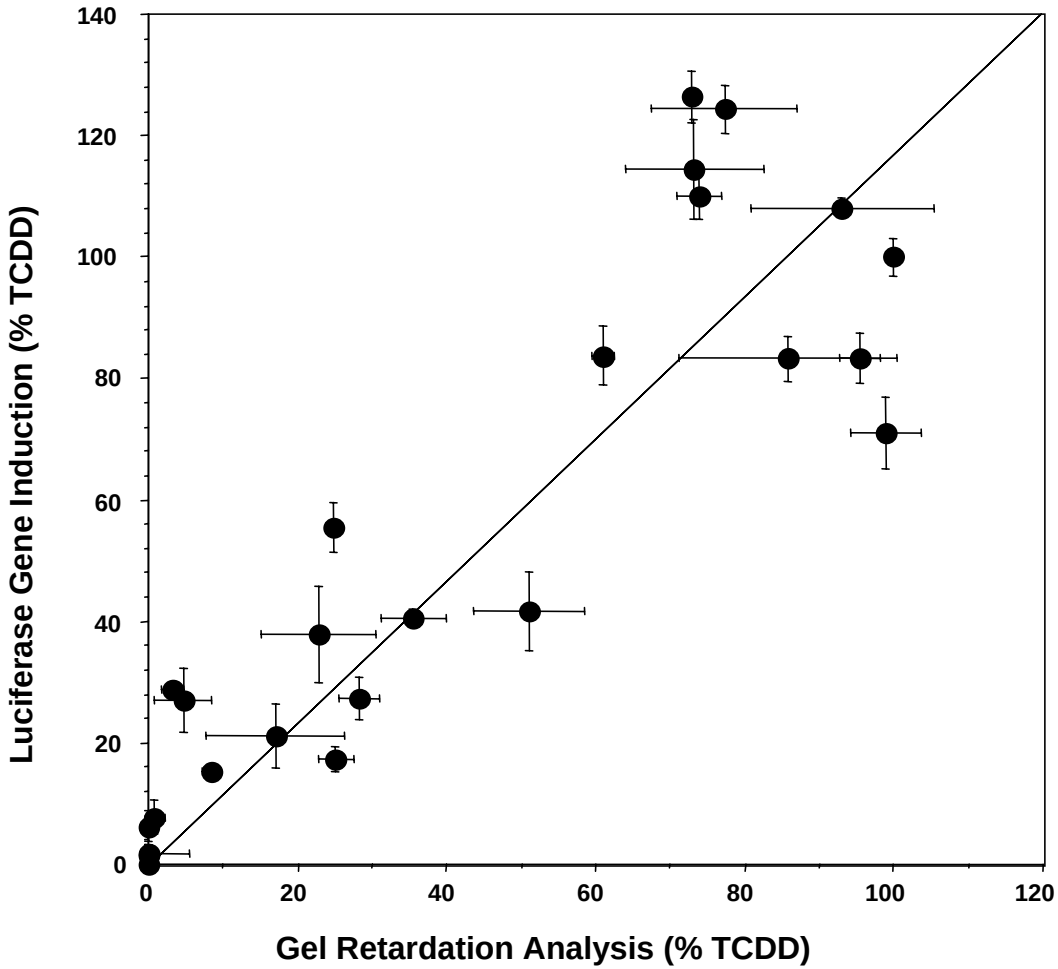
DMSO Extracts of Commercial and Consumer Products Contain Chemicals that Stimulate AhR DNA Binding



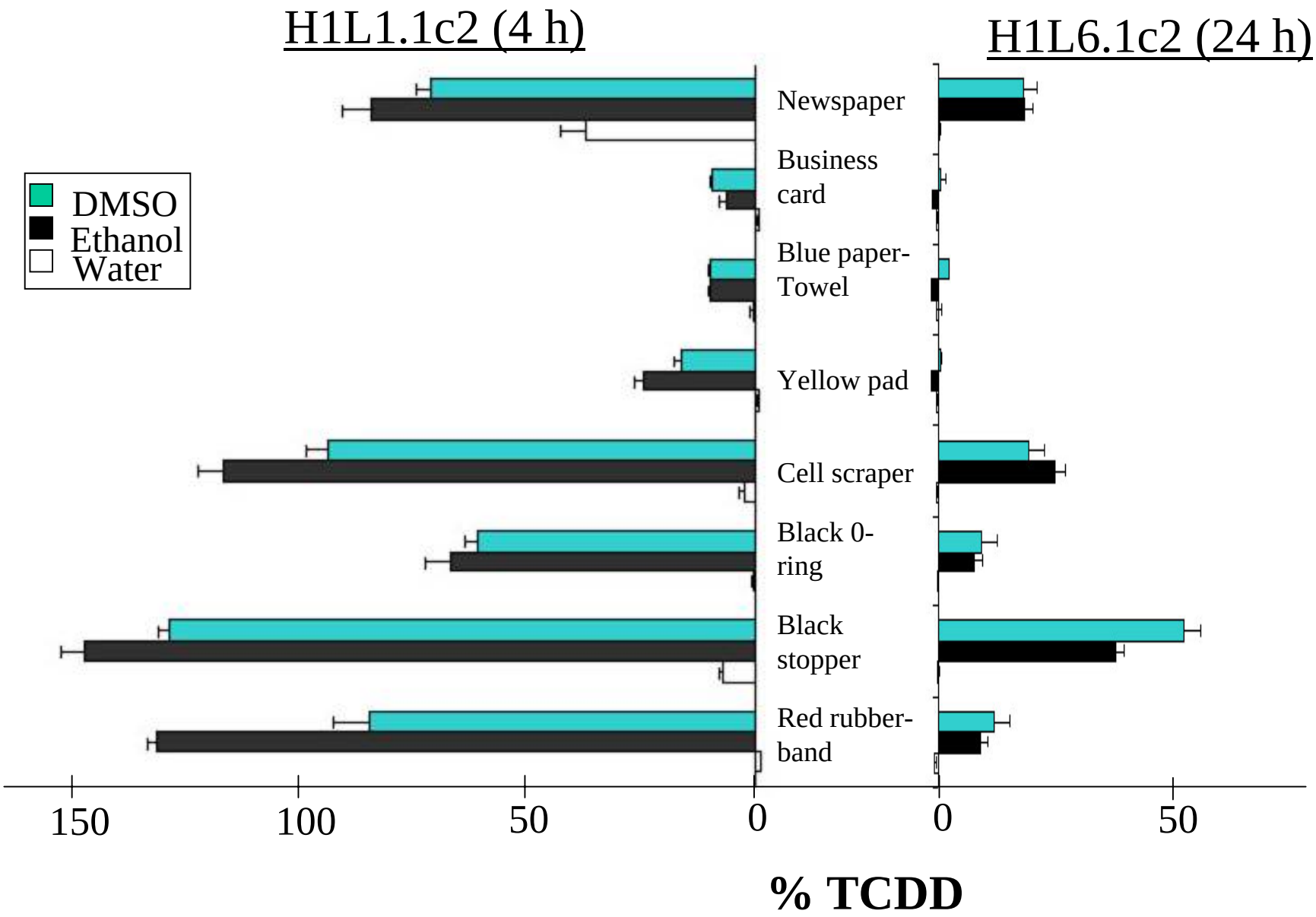
DMSO, Ethanol and Water Extracts of Selected Products Stimulate AhR DNA Binding



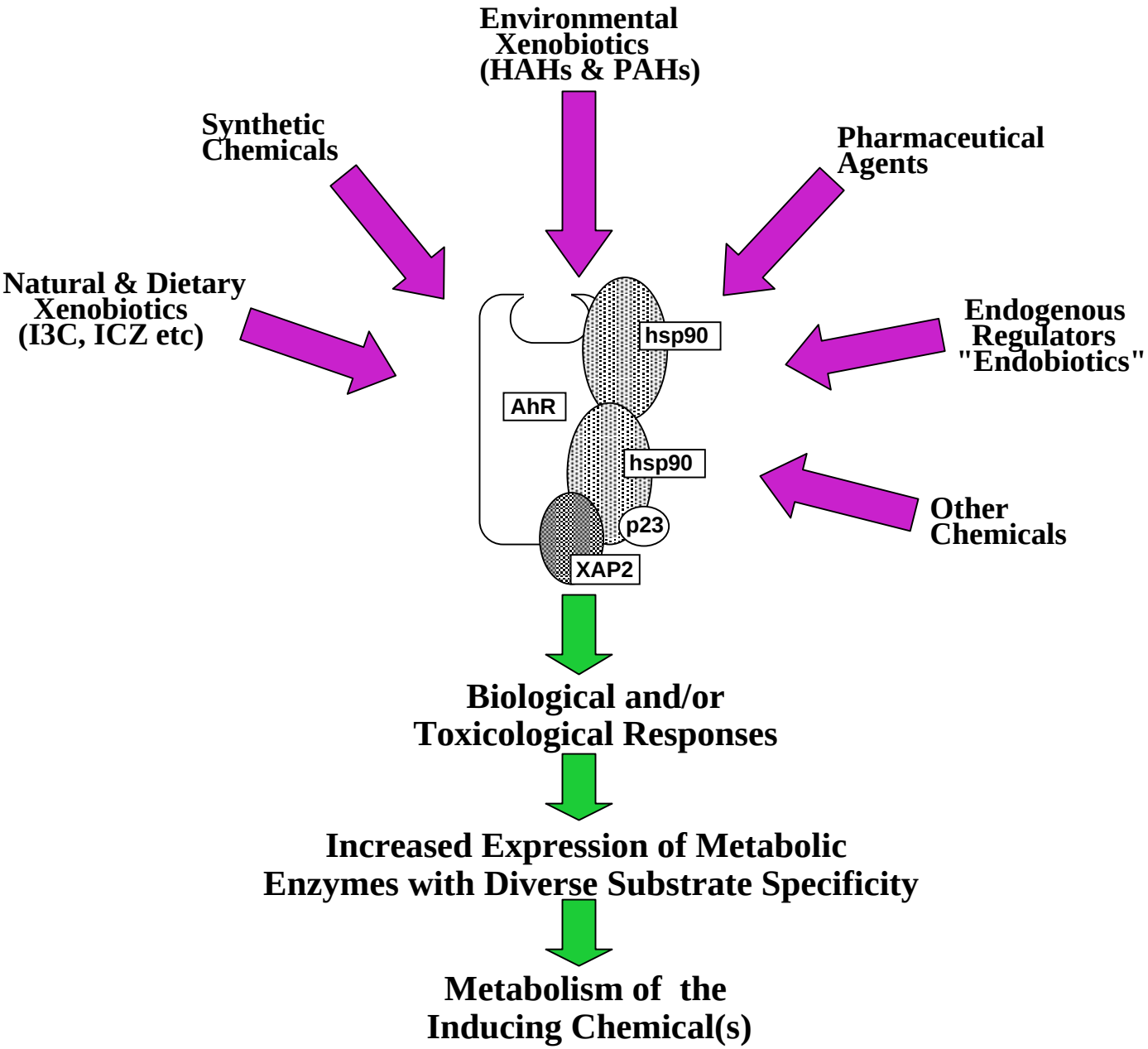
There is a Good Correlation Between the Ability of an Extract to Stimulate Guinea Pig AhR DNA Binding and Reporter Gene Expression



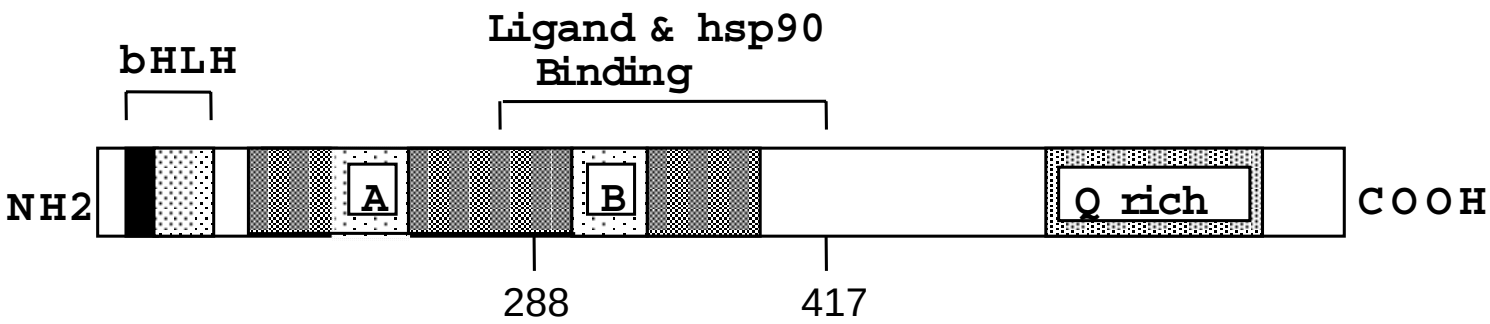
AhR Agonists in Commercial Products are Metabolically Labile



Activation of the Ah Receptor By Diverse Classes of Chemicals

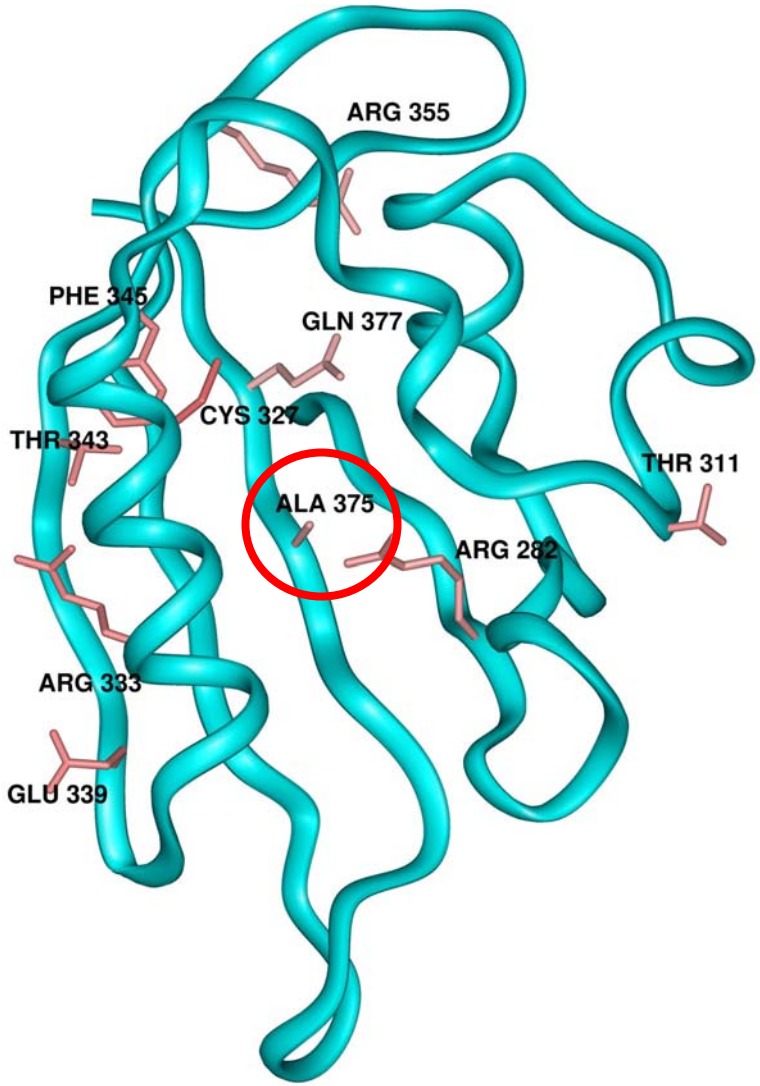


What are the Structural and Functional Determinants of the AhR Ligand Binding Domain?

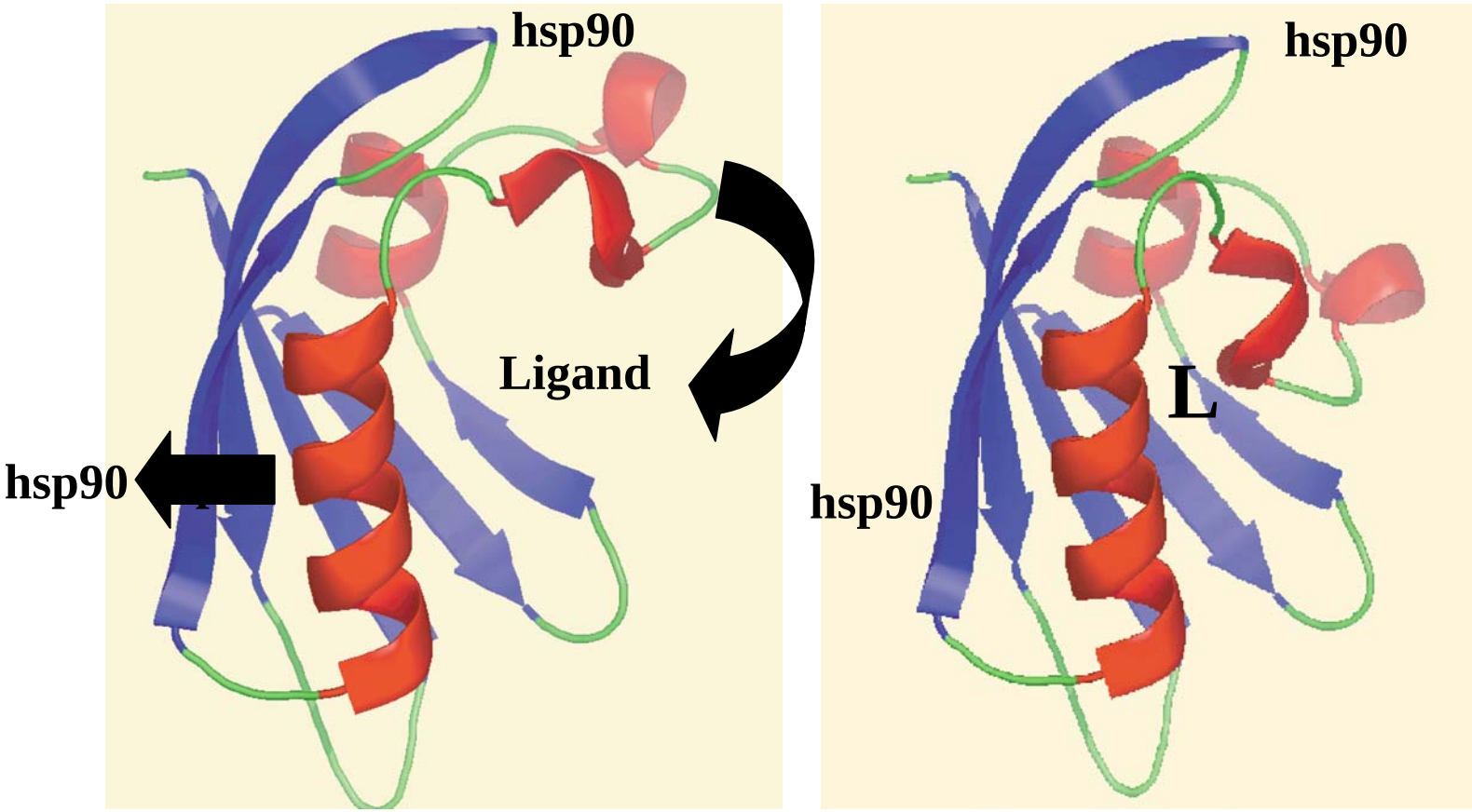


mAhR modeled region: PAS B Domain (aa 277 - 380)

Homology Model of the AhR Ligand Binding Domain



Hypothetical Ligand-Induced Conformational Change in the Ah Receptor Ligand Binding Domain



Conclusions I

The AhR can bind and be activated by a structurally diverse array of ligands and it plays a key role as an “environmental sensor” leading to enhanced metabolism and degradation of exogenous and endogenous chemicals.

Not all AhR agonists are created equal - while all can induce gene expression, not all can produce toxic effects. The toxic potency of an AhR agonist is primarily dependent on its metabolic stability and persistence in the cell.

The binding of ligand to the AhR (TCDD and β-naphthoflavone) is essentially irreversible, which invalidates current ligand binding affinity estimates and which assume equilibrium binding conditions. The measured binding affinities are actually ligand association rates.

Conclusions II

The structural diversity in AhR ligand binding is not surprising given the role of the AhR as an activator of expression of metabolic enzymes each of which can bind and metabolize a structurally diverse array of chemicals.

This diversity also implies that the AhR has a very promiscuous ligand binding pocket and suggests the existence of multiple endogenous physiological ligands.

QSAR modeling of structurally diverse AhR ligands and the AhR ligand binding domain will provide information with regards to the key structural and physiochemical characteristics important for AhR ligands and ligand binding.

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